Journal of Applied Botany and Food Quality 94, 213 - 219 (2021), DOI:10.5073/JABFQ.2021.094.026

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Correlation analysis of chlorogenic acid and luteoloside biosyntheses with transcription levels of HQTs and FNSs in *Lonicera* species

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(Submitted: June 16, 2021; Accepted: October 31, 2021)

Summary

Organic acids and flavonoids are the main active components in *Lonicera* species. Chlorogenic acid and luteoloside are important components, and their synthesis is regulated in plants by the phenylpropanoid synthesis pathway. Downstream of the phenylpropanoid synthesis pathway, hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (HQT) and flavone synthase (FNS) are critical enzymes that are involved in chlorogenic acid and luteoloside biosynthesis, respectively.

In this study, we first determined the dynamic accumulations of chlorogenic acid, luteoloside and other active components in different growth stages of the flower buds of *Lonicera fulvotomentosa* through HPLC-DAD and then investigated the expressions of the *LJHQT* and *LJFNS* gene families by q-RT-PCR. In addition, we also compared the expression levels of *HQT* and *FNS* orthologous genes in various tissues of *Lonicera japonica, L. fulvotomentosa, and Lonicera hypoglauca*.

The results indicated that the chlorogenic acid contents exhibit leaf accumulation that is preferential in *L. fulvotomentosa* but exhibit bud accumulation that is preferential in *L. japonica and L. hypoglauca*. The luteoloside contents show preferential leaf accumulation in these three species. Our results suggest that the leaves and buds of these three species are rich in medicinal ingredients, including chlorogenic acid (CGA) and luteoloside, and therefore can be used as a material to extract CGA and luteoloside rather than being wasted. Furthermore, combined with the transcript expression levels of *HQTs* and *FNSs*, we explained the species-specific and tissue-specific occurrence of CGA and luteoloside. We analyzed dynamic changes of components and gene expression and demonstrated that the expressions of *HQTs* and *FNSs* in these three species are closely related to the synthesis of chlorogenic acid and luteoloside.

Key words: *Lonicera* species; *HQT*; *FNS*; chlorogenic acid; luteoloside

Introduction

Lonicera species are widely distributed in China, among which twelve *Lonicera* species are used in traditional Chinese medicine (Li et al., 2018). According to the Chinese Pharmacopoeia, *Lonicera japonicae flos* (*LJF*) refers to the flower buds of *Lonicera japonica* (*LJ*), and *Lonicera flos* includes the flower buds of *Lonicera macranthoides* Hands.-Mazz. (*LM*), *Lonicera hypoglauca* Miq. (*LH*), *Lonicera confusa* DC. (*LC*), and *Lonicera fulvotomentosa* P.S. (*LF*). Hsu & S.C. Cheng (Chinese Pharmacopoeia Commission, 2020), which further promotes the essential value of relevant research in *Lonicera*.

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Lonicera plants exhibit antimicrobial, anti-inflammatory, antioxidation, antiviral, antipyretic, and antitumor activities (Shang et al., 2011); therefore, they are widely used as food raw materials and have health effects on the human body (Shang et al., 2011). The activity of *Lonicera* is mainly attributed to the abundant organic acids and flavonoids (Lee et al., 1995; Wu, 2007; Tang et al., 2008; Wang et al., 2009; Shang et al., 2011; Li et al., 2019), in which chlorogenic acid and luteoloside are the major effective components of organic acids and flavonoids, respectively (He et al., 2007; Chinese Pharmacopoeia Commission, 2020).

Biosynthesis of chlorogenic acid and luteoloside is controlled by the phenylpropanoid pathway (Yuan et al., 2014), which can be divided into two stages. In the first stage, p-coumaroyl-CoA is synthesized under the catalysis of L-phenylalanine ammonia-lyase (PAL), 4-coumarate CoA ligase (4CL), and cinnamate 4-hydroxylase (C4H) in sequence. In the second stage, p-coumaroyl-CoA is used as the universal precursor to synthesize CGA under the catalysis of hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (HQT) and to synthesize luteoloside under the catalysis of chalcone synthase (CHS), chalcone isomerase (CHI), flavone synthase (FNS), and a P450 modifying enzyme (Yuan et al., 2012). In the phenylpropanoid pathway, hydroxycinnamoyl-CoA quinate transferase (HQT) plays a key role downstream of the chlorogenic acid metabolic pathway (Mahesh et al., 2007; Comino et al., 2009). Furthermore, a similar regulatory effect on chlorogenic acid synthesis has been found not only in *LJ* (Peng et al., 2010; Kong et al., 2017) but also in tomato, coffee and other plants (Niggeweg et al., 2004; Sonnante et al., 2010; Navarre et al., 2013). On the other hand, flavone synthase (FNS) is the critical enzyme that is located downstream of luteoloside synthesis. In addition, the distributions of active components and related gene expressions in different parts of *LJ* show tissue specificity (Yuan et al., 2014; Li et al., 2018). It has been reported that flower buds have the highest expression levels of *HQTs* and chlorogenic acid contents; in contrast, stems and shoots have the lowest *HQT* expression levels and chlorogenic acid contents in *LJ* (Peng et al., 2010). Several articles have also revealed that different species of *LJ* exhibit differences in their chlorogenic acid and luteoloside contents (Li et al., 2018). In recent years, reports have focused on the differences among the components of *LJ* at different growth ages, species and origins (Li et al., 2018). Unfortunately, the relationships among enzyme activities and the expression levels of related genes and chemical compositions in *LJ*, *LH* and *LF* remain unknown.

In this study, the levels of the effective components, especially chlorogenic acid and luteoloside, at the different growth stages in *LF* were measured using HPLC-DAD, and physiological and biochemical methods were utilized to analyze the enzyme activities. By tracking the variations in chlorogenic acid and luteoloside contents and chan-

ges in the activities of their associated regulatory enzymes at different growth stages in *LF* and different parts of *LJ*, *LF* and *LH*, we systematically explained the effects of the enzyme activity changes on the dynamic accumulation of the main effective compounds. Moreover, the transcription levels of the *HQT* and *FNS* genes were detected by qPCR to explore the effects of the relevant genes on the accumulation of major components in the flower buds of *LF* throughout flower development. In addition, the differences in active component contents and expression levels of related genes in the buds and leaves of *LJ*, *LF* and *LH* are compared and discussed, which contribute to a better understanding of how genes influence the chemical compositions in different plants. In summary, our results provide a better way to understand the synthesis, accumulation and regulatory mechanism of chlorogenic acid and luteoloside in the *Lonicera* family.

Material and methods

Plant materials and sample collection

From March 28, 2014 to April 20, 2014, the experimental materials were collected from the buds and flowers of *LF* at different growth stages in the medicinal plant resource garden of South China Agricultural University (Fig. 1). The material was identified by Yang Qiner, a researcher from the South China Botanical Garden, Chinese Academy of Sciences, as *LF*. We selected new spring branches on the upper part of the *LF* plant and picked the buds or new flowers at six stages: young alabastrum (S1), green alabastrum (S2), slightly white alabastrum (S3), whole white alabastrum (S4), silvery flower (S5), and golden flower (S6) (Fig. 2).

Sample pretreatment: (A) Robust plants were selected, samples were collected at different growth stages, a microwave oven was used to change temperatures, samples were dried and they were placed in a drying oven for later use. (B) RNA extraction and enzyme activity sample determination: Fresh samples were collected, quickly treated with liquid nitrogen, and stored at -80 °C.

RNA extraction

Total RNA was extracted from plant tissues using TRIzol Reagent (Invitrogen, USA) and pretreated with RNase-Free DNase (Promega, USA) to eliminate genomic DNA contamination. RNA integrity was analyzed on 1% agarose gel. RNA quantities were determined using a NanoDrop 2000C Spectrophotometer (Thermo Scientific, USA). Total RNA was reverse-transcribed by Reverse Transcriptase MMLV (Takara, China).

PCR design and q-RT-PCR

The full lengths of the published series of CDSs, such as HQT and FNS, in *LF* were searched on the NCBI website, and Primer Premier 5 software (http://frodo.wi.mit.edu/primer 5/) was used to design the real-time PCR primers (Tab. 1).

Quantitative PCR system: 10 μL of 2×SYBR Green Premix Ex Tag, 0.5 μM of each primer, 5 μL of template cDNA (1000 ng/μL), and sterilized double-distilled water was added to reach a total volume of 20 μL.

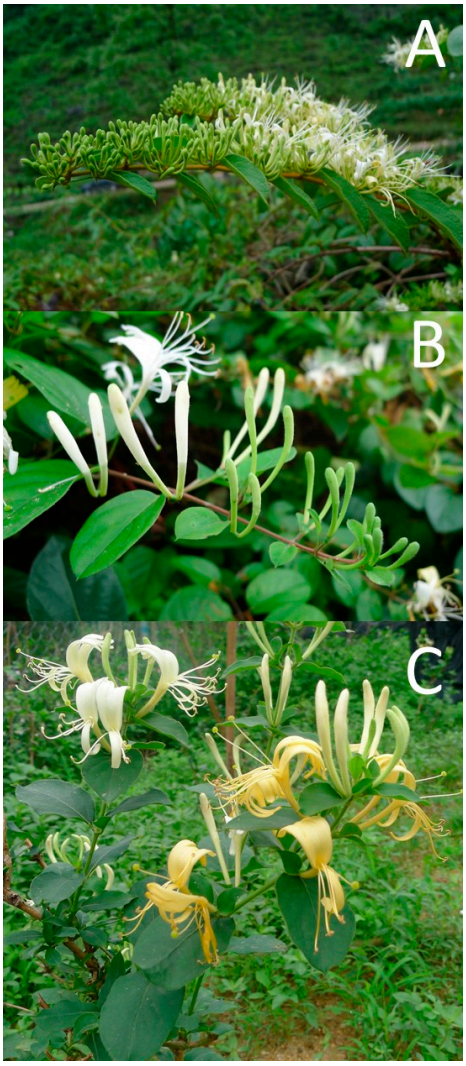


Fig 1: The flowers and leaves of (A) *L. fulvotomentosa,* (B) *L. hypoglauca* and (C) *L. japonica*.

Fig. 2: Morphological changes in the buds and flowers of *L. fulvotomentosa* at different growth stages from S1 to S6. S1: The stage of young alabastrum; S2: The stage of green alabastrum; S3: The stage of slightly white alabastrum; S4: The stage of whole white alabastrum; S5: The stage of silvery flower; S6: The stage of golden flower.

Tab. 1: The sequences of *HQT1*, *HQT2*, *FNS1* and *FNS2* primers.

gene	Forward	Reverse sequence
HQT1	TGAGATCCTAGCTGCCCACT	TGGCTGTGAACACCACATTT
HQT2	CAATCAAGTCCCAAGGCTGT	GGCAGCTAGGACCTCGTATG
FNS1	TCTCCCCATTAGGACCAACGA	CACCTCACGTACCAATGTCCT
FNS2	GCCACGGATACAACTGCGAT	TAGCCTGTGCTTCCCTACGA

Reaction procedure settings: Reactions were carried out at 95 °C for 3 min. Then, for 40 cycles, the reactions involved 15 s at 95 °C and 20 s at 72 °C, the degradation temperature was 55 °C and the reaction time was 30 s. The PCR products were analyzed using CFX Manager 2.0 software (Bio-Rad, United States). The actin gene expression quantities were chosen as an endogenous control for studying the gene expressions in buds of *LF*, and the relative expression results were obtained using the $2^{-\Delta\Delta CT}$ method (Livak et al., 2001). Each value is the average of three biological replicates.

HPLC - DAD detection

Based on the sample preparation method, samples from each period were divided into 3 pieces and were then passed through a 60-mesh sieve after crushing. By referring to the methods introduced by the Pharmacopoeia of The People's Republic of China (Chinese Pharmacopoeia Commission, 2020), 0.10 g of each sample was accurately weighed, this was placed in a 100 mL triangular bottle, 25 mL of 50% methanol was added and weighed, the solution was subjected to ultrasound at 50 °C (40 min power 150 W), the solution was cooled and weighed, and lost water was replenished. Ultrasound was applied three times. Then, the liquid was filtered through a 0.22 μm filtration membrane for content determinations by HPLC.

In this study, the samples were determined by HPLC (Agilent, 1260C, USA). The chromatographic workstation included an online vacuum degassing machine (G-1322A), high-pressure quaternary pump (G-1311A), standard automatic sampler (G-1313A), intelligent column temperature box (G-1316A), variable wavelength detector (G-1313A), diode array detector, Agilent 1260 series chromatographic workstation, CG-16 W high-speed microcentrifuge, and SB3200T ultrasonic cleaning instrument. Chromatographic conditions: chromatographic column: Agilent C18 (4.6 mm×250 mm, 5 μm); mobile phase: A (acetonitrile)-B (0.05% phosphoric acid aqueous solution); the flow rate was 0.5 mL·min-1. detector: Agilent 1260 DAD detector, wavelength: 240-327 nm; the column temperature was 25 °C, and the sample size was 5 μL. The gradient elutions were 0-21 min (10-17% A) and 21-33 min (17-23% A). The theoretical plate number was not less than 3000.

Preparation of the control sample and linear range investigation: chlorogenic acid (327-97-9, ≥98%) was purchased from Chengdu Munster Biotechnology (Chengdu, China). The 0.1, 0.2, 0.3, 0.5, and 0.7 mL chlorogenic acid control solutions (3 mg·mL^{-1}) were absorbed and placed in 5 mL volumetric flasks with 50% methanol and were shaken evenly to make 5 standard solutions with different concentrations, 5 μL was injected separately. Linear regression was carried out with the injection amounts of the standard solutions of the control substance (e.g., μg of chlorogenic acid) as the x-coordinate (X) and the peak area as the y-coordinate (Y).

Results

Contents of the main effective components and relevant gene expressions in leaves and buds of *LJ*, *LH* and *LF*

According to the Chinese pharmacopoeia, only *FLJ* (*L. japonica* flowers) at the golden and silver flowering stages have been harvested over a long period, and many leaves are often wasted. In addition, studies in recent years have found that other *Lonicera* species can be used as substitutes for *FLJ*, as those materials may also contain chlorogenic acid and luteoloside, which are the active compounds in *LJ*. Therefore, in this study, we measured the CGA and luteoloside contents in the buds (golden and silver flowers) and leaves of *LJ, LF, LH* and determined the relevant gene (*HQTs* and *FNSs*) expression levels. As shown in Fig. 3a, there was abundant chlorogenic acid in the leaves of *LJ*, but the chlorogenic acid content in the leaves was slightly lower than that in the buds. In addition, *LJ* and *LH* also accumulated high amounts of chlorogenic acid. The chlorogenic acid contents in both the flower buds and leaves of *LH* were significantly higher than those in *LJ*. The chlorogenic acid content in the leaves of *LF* was not only higher than that in the buds but was also higher than that in the leaves of *LJ*. As shown in Fig. 3b, the luteoloside content in the leaves of *LJ* was significantly higher than that in the flower buds. Although the luteoloside content in the leaves of *LF* was significantly higher than that in the flower buds, the luteoloside contents in both of them were significantly lower than that in *LJ*. For *LH*, only a very small amount of luteoloside accumulation was detected in the leaves, and no luteoloside was detected in the flower buds.

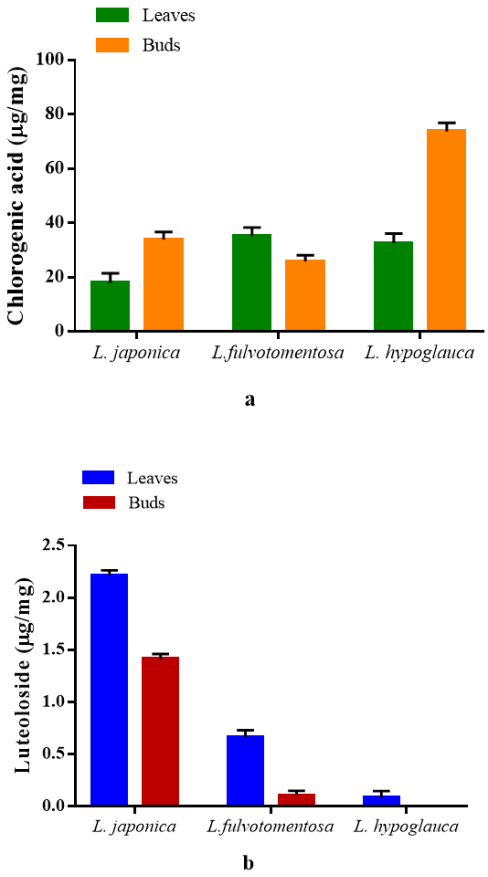


Fig. 3: Contents of CGA and luteoloside in *LJ, LF, and LH*. The contents of chlorogenic acid (a) and luteoloside (b) in two different parts of the flower buds and leaves of the three species at the golden flowering stage and silver flowering stage that were detected by HPLC.

Associated gene transcription in the leaves and buds of *LJ*, *LH*, and *LF*

HQT gene family

Hydroxycinnamoyl-CoA quinate: hydroxycinnamoyl transferase (HQT) is an enzyme involved in chlorogenic acid biosynthesis is that exhibits catalytic activity in the esterification of quantic acid with

caffeoyl-CoA to generate CGA (YUAN et al., 2012). Through quantitative analysis, there were no significant differences in the *HQT1* transcription levels between the flower buds and leaves of *LJ,* while the *HQT2* expression level in leaves was lower than that in buds (Fig. 4a and b). For *LH, HQT1* and *HQT2,* the transcription levels were higher in the leaves, and the transcription level of *HQT1* in the leaves of *LH* was significantly higher than that in the leaves of *LJ*. However, the transcription level of *HQT1* in the flower buds of *LH* was lower than that in the flower buds of *LJ*, but the transcription level of *HQT2* in the flower buds of *LH* was higher than that in the flower buds of *LJ*. Overall, the *HQT1* expression level in the buds of *LJ* was higher than those in *LF* and *LH*, but the *HQT1* and *HQT2* expression levels in the leaves of *LJ* were lower than those in *LF* and *LH*. To our surprise, the *HQT2* expression level in the buds of *LH* was also higher than that in *LJ*, which means that the *HQT2* expression levels in both the buds and leaves of *LH* were higher than the *HQT2* expression levels in other species.

FNS gene family

The *FNS1* and *FNS2* expression levels in the leaves of *LJ*, *LF and LH* were significantly higher than those in flower buds (Fig. 4c and d). The expression level of *FNS1* in the leaves of *LJ* was higher than that in LH but was lower than that in *LF*. In addition, the expression level of *FNS2* in LJ leaves was not different from that in *LH* leaves but was also lower than that in *LF* leaves. This difference was observed when comparing the expression levels of *FNS1* and *FNS2* in buds among *LJ*, *LF* and *LH*. The expression levels of *FNS1* and *FNS2* in the flower buds in *LJ* were significantly higher than those in *LF* and *LH*. The expression level of *FNS1* in LF buds was higher than that in LH buds. Meanwhile, the expression levels of *FNS2* showed no differences between *LF* and *LH* buds.

Dynamic accumulation of the main effective components in *L. fulvotomentosa* at different growth stages

The chemical compositions of flower buds in *LF* at different growth stages were determined by HPLC-DAD. *LF* consists mainly of organic acids and flavonoids.

In the flower buds of *LF,* the CGA contents were significantly higher than the luteoloside contents in each stage (Fig. 5a). There were also some differences in the accumulation regularity between the CGA and luteoloside contents. For example, the CGA content first increased and then decreased. Compared to the CGA content in S1 (21.52 μg/mg), the CGA content in S2 (25.26 μg/mg) increased and reached its highest value, whereas the CGA contents in S3 (25.85 μg/mg), S4 (25.78 μg/mg) and S2 were not significantly different. Then, in S5 and S6, the CGA contents (24.55 μg/mg and 25.01 μg/mg, respectively) showed no significant difference but were remarkably lower than the CGA content in S4 but were still higher than the CGA content in S1.

The luteoloside contents exhibited a decreasing trend during S1-S5. The luteoloside content in S2 (0.24 μg/mg) was significantly lower than that in S1 (0.31 μg/mg), and statistical analysis showed that there were no significant differences in luteoloside contents among S2 (0.24 μg/mg), S3 (0.23 μg/mg), S4 (0.20 μg/mg), S5 (0.14 μg/mg), and S6 (0.16 μg/mg) (Fig. 5a).

The neochlorogenic acid contents increased slightly from S1 to S4 and then significantly decreased in S5 but markedly increased in S6. *LF* had the lowest neochlorogenic acid content in S5 (1.14 μg/mg), which was similar to that in S1 (1.12 μg/mg), whereas it peaked in S6 (1.23 μg/mg), which exhibited a significant difference from the other stages ($P<0.05$) (Fig. 5b).

From S1 to S6, cryptochlorogenic acid exhibited a "double peak" trend. The cryptochlorogenic acid content peaked at S4 (0.92 μg/mg) and was followed by S2 (0.90 μg/mg), and the cryptochlorogenic acid

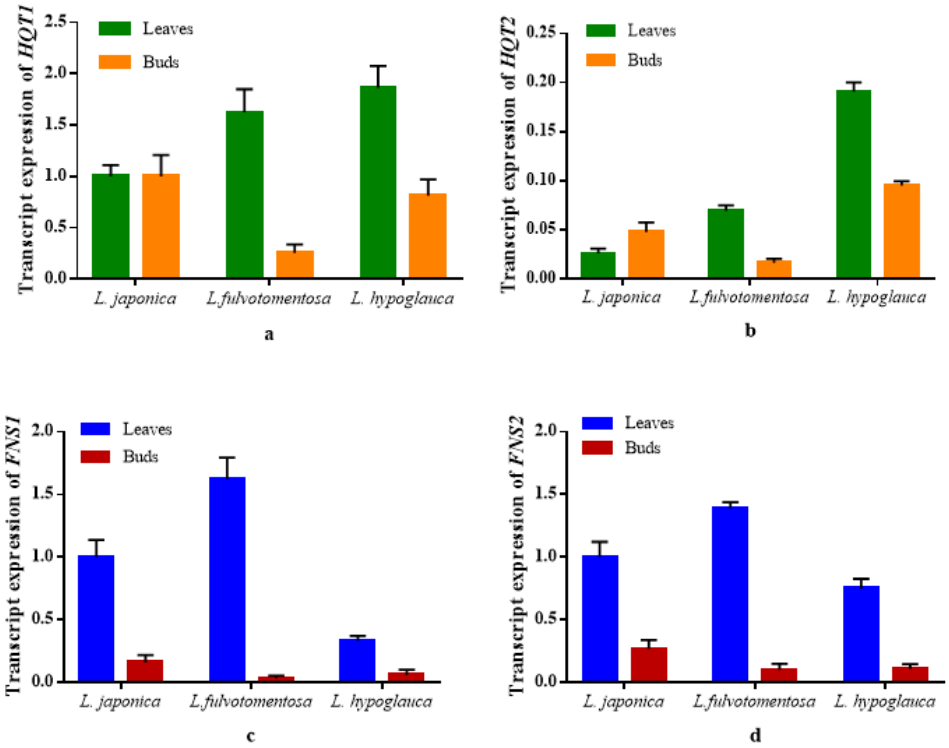


Fig. 4: The transcript expression levels of *HQT1* (a), *HQT2* (b), *FNS1* (c), and *FNS2* (d) in two different parts of the flower buds and leaves of the three species at the golden flowering stage and silver flowering stage were detected by q-RT PCR.

content in S6 (0.73 μg/mg) was the lowest and was approximately 79% of that in S4 and 80% of that in S2 (Fig. 5c).

The caffeic acid content exhibited a gradually increasing trend from S1 to S6. Compared with the lowest caffeic acid content in S1 (0.023 μg/mg), the caffeic acid content was highest in S6 (0.029 μg/mg), which was approximately 1.26 times that in S1 (Fig. 5d).

Isochlorogenic acid A showed a gradually decreasing trend from S1 to 5 and then increased back to the S4 level in S6. The isochlorogenic acid A content peaked at S1 (73.17 μg/mg), similar to that in S2 (65.83 μg/mg) and S3 (69.19 μg/mg). The isochlorogenic acid A content was lowest in S5 (54.44 μg/mg), which was 74% of that in S1 (Fig. 5 g). The isochlorogenic acid C content showed a gradually decreasing trend from S1 to S6. The isochlorogenic acid C contents were highest in both S1 (33.20 μg/mg) and S2 (32.93 μg/mg), whereas *LF* had the lowest isochlorogenic acid C content of only 17.85 μg/mg in S6, which was approximately 54% of that in S1 (Fig. 5h). Statistical analysis showed that the contents of rutin and hyperoside did not change significantly from S1 to S6.

Dynamic accumulation of gene expression in *HQT* and *FNS* families in *FLF* at different growth stages

The expression levels of the *HQT* and *FNS* gene family members in the flower buds of *LF* from S1 to S6 were measured by qRT-PCR. The results (Fig. 6) showed that the expression trends in both *HQT1* and *HQT2* decreased from S1 to S6. The peak *HQT1* expression occurred in S2, while the peak *HQT2* expression appeared in S1, and both *HQT1* and *HQT2* expression fell to their lowest points in S6.

After statistical analysis, there were no significant differences in the expression of *HQT1* between S1 and S2, which proved that the expression of *HQT1* reached its peak at S1 and S2 simultaneously. However, the expression of *HQT1* decreased by approximately 10% from the peak value in the S3 phase. After that, the expression of *HQT1* decreased continuously and reached the lowest value in S5, with no significant difference in S6. Furthermore, the expression of *HQT2* was slightly different from that of *HQT1*. The lowest expression levels of *HQT1* in S5 and S6 were approximately 65% lower than the peak values in S1 or S2. The expression of *HQT2* in the S2

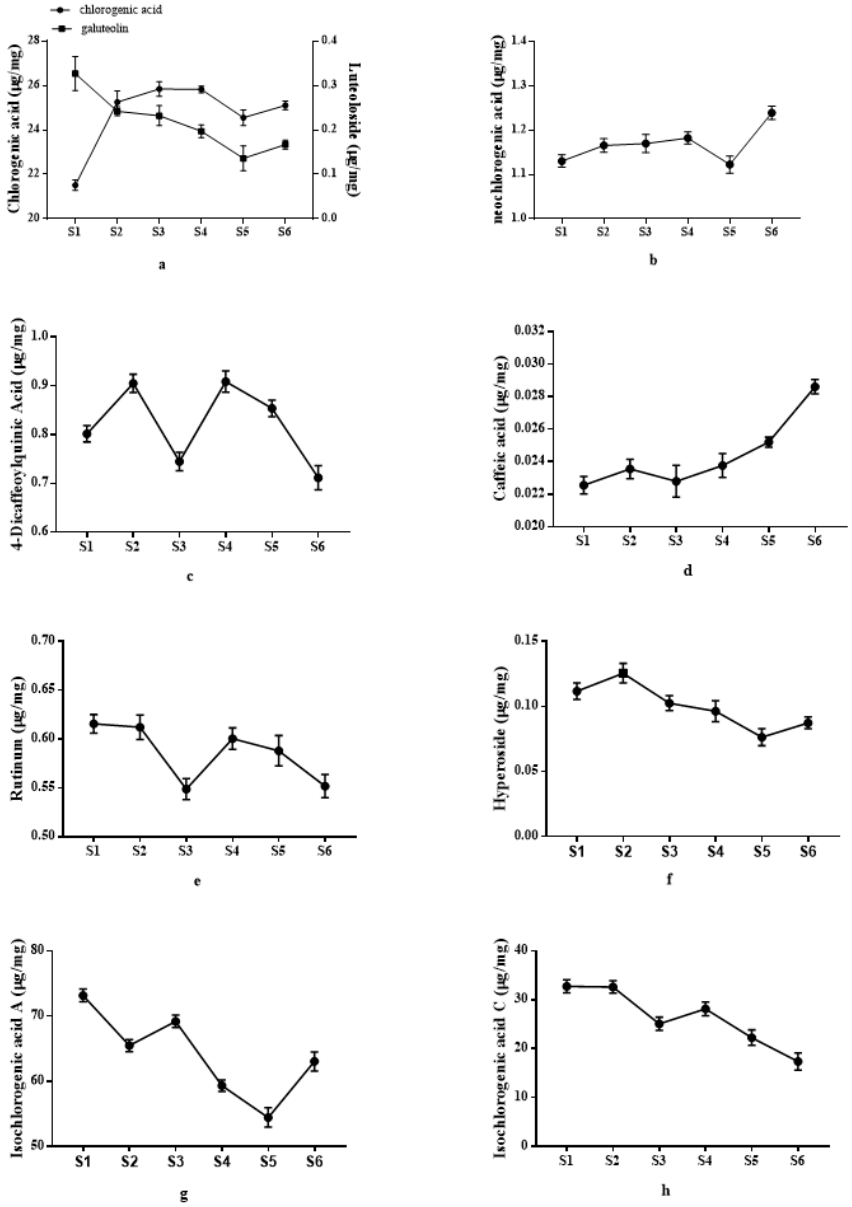
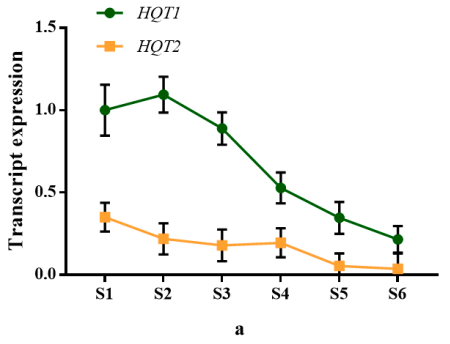


Fig. 5: Dynamic accumulations of chlorogenic acid and luteoloside (a), neochlorogenic acid (b), 4-dicaffeoylquinic acid (c), caffeic acid (d), rutin (e), hyperoside (f), isochlorogenic acid A (g), and isochlorogenic acid C (h) in the flower buds of *L. fulvotomentosa* at different growth stages from S1-S6.

stage was reduced by 38% compared with that in S1. After that, the expression of *HQT2* was relatively stable in the S2, S3 and S3 phases and showed no significant changes. Until the S5 stage, the expression level of *HQT2* showed a significant decrease, and the expression level for the S6 stage no longer showed a significant decrease. The lowest expression levels of *HQT2* in S5 or S6 were approximately 84% lower than that in S1.

FNS1 showed a significant decrease (17%) from S1 to S2. Notably, after statistical analysis, the subsequent *FNS1* expression did not show a significant difference. In addition, the expression levels of *FNS2* showed no significant differences between the S1 and S2 stages but decreased by approximately 69% in the S3 stage compared with before. There was no significant change in the expression level of *HQT2* in S4 phase. However, the expression of *HQT2* in S5 was significantly lower by approximately 45% compared with that in S4. In addition, *HQT2* increased by approximately 84% in S6 compared with S5. In general, the expression of *FNS1* was highest in the S1 stage. The expression of *FNS2* was highest in S1 and S2.



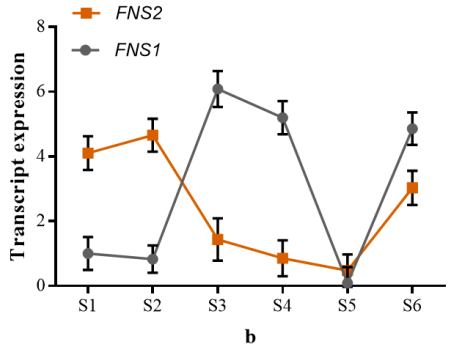


Fig. 6: Transcript expression levels of *HQT* family members, including *HQT1* and *HQT2* (a), *FNS family members,* including *FNS1* and *FNS2* and (b) at different growth stages of the flower buds of *L. fulvotomentosa*.

Discussion

Tissue-specific accumulation and species-specific accumulation of CGA and luteoloside

According to the description from the China Pharmacopoeia Commission, the traditional Chinese medicine JINYINHUA means the dry flowers of *LJ* in the silver stage (S5) and golden stage (S6), and the main essential bioactive chemical compounds consist of chlorogenic acid and luteoloside (Chinese Pharmacopoeia Commission; 2020). Currently, many studies are focused on confirming the location and accumulation of bioactive compounds with developmental stages (Li et al., 2009; Yuan et al., 2014; Wang et al., 2019). Moreover, most researchers prefer to use *LJ* as the research material and ignore the other two species. Therefore, some articles have already shown that chlorogenic acid and luteoloside also exist in the leaves of *LJ* and *LF* (Chen et al., 2010), but the differences in chlorogenic acid and luteoloside contents, as well as the differences in related gene expression levels between leaves and flowers, are not compared in detail.

In this study, the compositions of the flower buds and leaves of three *Lonicera* species from S5 to S6 were analyzed by HPLC. *LJ* and *LH* showed preferential accumulation of CGA in flower buds, while preferential accumulation of CGA in leaves was observed in *LF* (Fig. 3a). Moreover, luteoloside preferentially accumulated in the leaves of the three *Lonicera* species (Fig. 3b). These results suggest that the leaves have certain medicinal value and should be properly harvested and utilized rather than discarded.

Next, we further analyzed the transcriptional levels of *HQTs* and *FNSs*, both of which have two gene family members (Yuan et al., 2012). As shown in Fig. 4a and 4b, for *LJ*, there were no significant differences in the expression levels of *HQT1* in buds and leaves, but the expression levels of *HQT2* were higher in flower buds than in leaves, which is consistent with the fact that more chlorogenic acid accumulates in the buds. These results indicate that the higher *HQT2* transcription levels in the flower buds of *LJ*, are correlated with the higher accumulations of chlorogenic acid in the flower buds of *LJ*. In addition, for *LF*, the higher chlorogenic acid accumulations in leaves are associated with higher transcription levels of *HQTs* in leaves, which again illustrates the correlation between *HQTs* and chlorogenic acid expression. However, the changes in the CGA and *HQT* expression levels in *LH* were not completely consistent, and the causes for this difference are still unclear. We speculate that *HQT* expression of chlorogenic acid occurs earlier than harvest time. In addition, all three *Lonicera* species show a preference for luteoloside accumulations in leaves, which is related to the higher *FNS* transcription levels in leaves.

Correlation between *HQT* and *FNS* transcripts and accumulations of chlorogenic acid and luteoloside

A number of articles have reported that *HQTs* are directly related to CGA synthesis and accumulation in *LJ*. Simultaneously, it has been reported that *FNSs* are involved in luteoloside synthesis and accumulation in *LJ* (Yuan et al., 2014). Our research indicates that the expression of HQT genes is also involved in the synthesis and accumulation of CGA in *LF,* and *HQT1* gene expression may be directly related to CGA accumulation. The results showed that when the accumulation of CGA synthesis begins, HQT genes will be expressed to participate in the process of CGA synthesis, and when the accumulation of CGA content stops or even decreases after reaching a certain level, the expression levels of *HQTs* will significantly decrease. Although *HQT2* gene expression may not fully match the accumulation of CGA, it may be caused by species deviations or *HQT2* genes may be redundant with *HQT1*.

On the other hand, the luteoloside contents in the leaves and flowers of *LJ* were significantly higher than those in the other two species. Furthermore, the luteoloside content is extremely low in leaves and was not detected in the flowers of *LH,* which indicated that there are differences in luteoloside contents among *Lonicera* species. Unlike *LJ,* the *FNS* gene expression levels in the flower buds of *LF* during different developmental stages do not match the synthesis and accumulation of luteoloside. A possible explanation is that the luteoloside content in *LF* is much lower (over 10 times) than that in *LJ*. Therefore, the expression of *FNSs* is also at a very low level in the flower buds of *LF,* which may lead to a trend of unstable expression and cannot match the change of luteoloside contents in flower development of *LF*. In addition, all three *Lonicera* species prefer to accumulate luteoloside in their leaves, which is related to the higher *FNS* transcription levels in leaves.

Conclusion

In conclusion, our study demonstrated that the synthesis and accumulation of chlorogenic acid and luteoloside were species-specific and tissue-specific and were related to the regulation of *HQT* and *FNS* gene expression levels. The synthesis and accumulation of chlorogenic acid and luteoloside in *LF* were correlated with the expression levels of *HQTs* and *FNSs*. Our research results provide a basis for developing *LF* and *LH* as substitutes for *LJ* and rational harvesting of leaves as Chinese medicinal materials. At present, many studies have focused on determining the locations, accumulations and development stages of bioactive compounds in *LJ* (Li et al., 2009; Yuan et al., 2014; Wang et al., 2019) and have ignored the other two species. Our results address the research gap in this area for *LF*. In addition, another innovation of our study is to compare the content differences of chlorogenic acid and luteoloside in the leaves and flower buds of three *Lonicera* plants, as well as the related gene expression differences. However, future work is therefore necessary to combine proteomic studies to explain the content differences and gene expression differences.

Acknowledgements

The authors thank Mr. Changjun Zhou of Longlin DaHongBao Lonicera Development Co., Ltd. for providing the original materials. This study was supported by the Natural Science Foundation of Guangdong Province (2019A1515011005), the National Natural Science Foundation of China (31500261) and the GuangDong Basic and Applied Basic Research Foundation, China (2020B1515420007).

Conflict of interest

No potential conflict of interest was reported by the authors.

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